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**Lobular patterns of cerebellar resting-state connectivity in adults with Autism Spectrum Disorder**

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## Abstract

Autism Spectrum disorder is a neurodevelopmental disorder characterized by core deficits in social functioning. Core autistics traits refer to poor social and imagination skills, poor attention-switching/strong focus of attention, exceptional attention to detail, as expressed by the Autism-Spectrum Quotient. Over the years, the importance of the cerebellum in the etiology of Autism Spectrum Disorder has been acknowledged. Neuroimaging studies have provided a strong support to this view, showing both structural and functional connectivity alterations to affect the cerebellum in Autism Spectrum Disorder. According to the underconnectivity theory, disrupted connectivity within cerebello-cerebral networks has been specifically implicated in the etiology of Autism Spectrum Disorder. However, inconsistent results have been generated across studies. In the present study an integrated approach has been used in a selected population of adults with Autism Spectrum Disorder to analyze both cerebellar morphometry and functional connectivity. In individuals with Autism Spectrum Disorder, a decreased cerebellar grey-matter volume affected the right Crus II, a region showing extensive connections with cerebral areas related to social functions. This grey matter reduction correlates with the degree of autistic traits as measured by Autism Spectrum Quotient. Interestingly, altered functional connectivity was found between the reduced cerebellar Crus II and contralateral cerebral regions, such as frontal and temporal areas. Overall the present data suggest that adults with Autism Spectrum Disorder present with specific cerebellar structural alterations that may affect functional connectivity within cerebello-cerebral modules relevant to social processing and account for core autistics traits.

## 1. Introduction

Autism Spectrum Disorders (ASD) is a neurodevelopmental condition typically characterized by deficits in social communication and interaction and repetitive behavior or restricted interests (Diagnostic and Statistical Manual of Mental Disorders–Fifth Edition [DSM-5], American Psychiatric Association, 2013). It has been evidenced that brain abnormalities play an important role in the development of ASD and involve both grey matter (GM) and white matter (WM) in frontal, temporal, parietal areas (Abell et al., 1999; Carper et al., 2002; Hazlett et al., 2006; Minshew and Williams, 2007) as well as subcortical structures (Sparks et al., 2002; Amaral et al., 2008; Cauda et al., 2011). Among the latter ones, the cerebellum has been consistently implicated in the ASD etiology, since that cerebellar GM abnormalities emerged as one of the biomarkers to discriminate individuals with ASD from typically developing individuals (Ecker et al., 2010). Neuroimaging studies have supported the evidence of a cerebellar involvement in ASD

(Courchesne et al., 1994, 1997; Rojas et al., 2006; Riva et al., 2013). Indeed, specific volumetric alterations of the cerebellum have been reported (Stoodley, 2014; D’Mello et al., 2015), mainly converging on the right cerebellar lobule VII (Crus I/II), a region considered to be part of the social cognitive cerebellum (Van Overwalle et al., 2014, 2015a,b, 2016; Van Overwalle and Marien, 2016; Stoodley and Schmahmann, 2009). In particular, **fMRI studies** in healthy subjects have demonstrated a direct role of the cerebellum in social mentalizing (Van Overwalle et al., 2014; 2015a,b) and functional connectivity between mentalizing cerebellar areas, converging onto right posterior lobule, and well-known mentalizing areas in the cerebrum (Van Overwalle et al., 2016; Van Overwalle and Marien, 2016). Furthermore, functional connectivity (FC) abnormalities between **the cerebellum** and **the cerebral cortex** regions of the social brain have been described in ASD individuals (Khan et al., 2015), involving also the dentate nucleus (Olivito et al., 2016). The underconnectivity theory (Just et al., 2004) has been proposed as an explanatory model of ASD and it attributes the impairment in psychological functions such as Theory of Mind (ToM) and executive processing to disrupted connectivity within different functional networks, particularly involving frontal and posterior regions in the cerebral cortex (Assaf et al., 2010; Redcay et al., 2013; Washington et al., 2014). It is worth noting that the contribution of the cerebellum to these functional networks has been widely evidenced in healthy subjects (Allen et al., 2005; Habas et al., 2009; Bernard et al., 2012), showing in particular right **Crus I/II** to have structural and functional connectivity with fronto-parietal and default mode networks (DMN) (Hoover and Strick, 1999; Kelly and Strick, 2003; Strick et al., 2009; Buckner et al., 2011; D’Mello et al., 2015), that are strictly related to **cognitive functions and social abilities**. According to this evidence, a correlation between GM differences in the cerebellar Crus I/II and core **ASD** profile has been found (D’Mello et al., 2015). The idea is that atrophied cerebellar regions may impact long-distance regions in the cerebral cortex by impeding the outflow of the cerebellar cortex to certain social brain regions. Thus, cerebellar GM differences could result in altered connectivity with multiple cortical areas associated to social behavior and affect cerebro-cerebellar interaction.

However, it has to be considered that the results from ASD studies have been apparently inconsistent across literature. This variability may be due to the heterogeneity of ASD. Indeed, subsamples of children, adolescents, and adults with ASD have been shown to present distinct patterns of brain alterations (Lin, et al., 2015). In spite of this discrepancy among studies, the neuroimaging data by structural and functional MRI have meaningfully generated insight in our understanding of ASD (for a review see Stigler et al., 2011).

Within this framework, integrated MRI studies of morphometry and functional connectivity in adults with high-functioning ASD are still lacking. In light of these observations, in the present study we aimed to investigate both structural and functional patterns of alteration in a sample of high-functioning adult individuals with ASD. To this aim voxel-based morphometry (VBM) was first used to detect the pattern of structural alteration affecting the cerebellum of ASD individuals. Subsequently, the patterns of functional connectivity of atrophied cerebellar regions **have been analyzed** by means of resting-state functional Magnetic Resonance Imaging (RS-fMRI), a useful MRI technique for investigating brain functional connectivity between anatomically separated brain regions (Biswal et al., 1997). By using this integrated approach, it is possible to investigate more accurately whether cerebellar GM differences associated with ASD may impact functional connectivity within specific cerebello-cortical networks thus accounting for ASD-related traits.

## **2. Material and Methods**

### **2.1 Subjects**

Ten adults [mean(SD) age=25.4 (9); M/F=5/5] with ASD were recruited for the current study, whose diagnosis had been assured prior to the inclusion by experienced psychiatrists and psychologists using gold standard diagnostic instruments for autism [Autism Diagnostic

Observation Schedule (ADOS) (Lord et al., 1989)] [mean(SD) 10.8 (3.1)]. ADOS scores fell within the clinical criteria for ASD ( $\geq 7$ ).

Secondary inclusion criteria included an estimated Intelligence Quotient (IQ) above 70 (mean(SD):113 (19.6) based on the Wechsler Adult Intelligence Scale-Revised (WAIS-R) (Orsini and Laicardi, 1997), and an absence of MRI-contraindications.

The Autistic Spectrum Quotient (AQ) (Baron-Cohen et al., 2001) was also administered showing the presence of clinically significant levels of autistic traits (mean/SD: 39.3 /7.2). **Demographic and clinical characteristics of ASD subjects are reported in Table 1.** All participants completed a comprehensive neurological examination performed by an expert neurologist. Participants with ASD did not take any medication at the time of testing and, according to the inclusion criteria, the absence of any neurological or psychiatric comorbidities was ensured. In addition, 23 typically developing adults (TDAs) [mean(SD) age = 25.5 (4.2); M/F = 14/9] with no history of psychiatric or neurological illness were enrolled as control group. The mean age of ASD patients and TDAs did not significantly differ as evidenced by the t-test analysis ( $t$ -value= 0.222  $p$ = 0.82 ) as well as the gender distributions among groups as evidenced by *Chi-square* test ( $X^2$ =0.34,  $df$ =1,  $p$ = 0. 56).

This research study was approved by the Ethics Committee of Santa Lucia Foundation, according to the principles expressed in the Declaration of Helsinki. Written informed consent was obtained from all subjects before study initiation.

### 2.3 MRI acquisition protocol

All subjects underwent an MRI examination at 3T (Magnetom Allegra, Siemens, Erlangen, Germany) that included the following acquisitions: 1) dual-echo turbo spin echo [TSE] (TR = 6190 ms, TE = 12/109 ms); 2) fast-FLAIR (TR = 8170 ms, 204TE = 96 ms, TI = 2100 ms); 3) 3D Modified Driven Equilibrium Fourier Transform (MDEFT) scan (TR = 1338 ms, TE = 2.4 ms,

Matrix =  $256 \times 224 \times 176$ , in-plane FOV =  $250 \times 250$  mm<sup>2</sup>, slice thickness = 1 mm); 3) T2\* weighted echo planar imaging (EPI) sensitized to blood oxygenation level dependent imaging (BOLD) contrast (TR: 2080 ms, TE: 30 ms, 32 axial slices parallel to AC-PC line, matrix:  $64 \times 64$ , pixel size:  $3 \times 3$  mm<sup>2</sup>, slice thickness: 2.5 mm, flip angle: 70°) for resting state fMRI. BOLD echo planar images were collected during rest for a 7 min and 20 s period, resulting in a total of 220 volumes. During this acquisition, subjects were instructed to keep their eyes closed, not to think of anything in particular, and not to fall asleep. The TSE scans of patients, acquired as part of this research study, were reviewed by an expert neuroradiologist in order to characterize the brain anatomy and determine the presence of macroscopic structural abnormalities. For the TDAs, conventional MRI was inspected in order to exclude any pathological conditions according to the inclusion criteria.

## 2.4 Image processing and data analysis

### 2.4.1 Voxel-based morphometry

Voxel based morphometry (VBM) was used to identify differences between ASD and TDAs in regional cerebellar volume. The cerebellum was pre-processed individually using the Spatially Unbiased Infratentorial Template (SUIT) toolbox (Diedrichsen et al., 2009) implemented in Statistical Parametric Mapping [Wellcome Department of Imaging Neuroscience; SPM-8 (<http://www.fil.ion.ucl.ac.uk/spm/>)]. The procedure involved: cropping and isolating the cerebellum from the T1 anatomical images; normalizing each cropped image into SUIT space; reslicing the probabilistic cerebellar atlas into individual subject space using the deformation parameters from normalization. The modulated GM probability maps were finally smoothed using a 8-mm FWHM Gaussian kernel and statistical analyses were performed on the resulting grey matter (GM) maps entered into a voxel-wise two-sample t-test analysis for assessing between group differences in

regional GM cerebellar volumes. Age and sex were set as variables of no interest. Results were considered significant at  $p$  values  $<0.05$  after FWE cluster-level correction (clusters formed with  $p < 0.005$  at uncorrected level). Additionally, every participant's MDEFT was also segmented in SPM in order to estimate the total grey matter (GM) volume and exclude the presence of macroscopic atrophy in ASD compared to TDAs.

#### 2.4.2 Resting-state fMRI data preprocessing

fMRI data were pre-processed using SPM8 (<http://www.fil.ion.ucl.ac.uk/spm/>), and in-house software implemented in Matlab (The Mathworks Inc, Natick, Massachusetts, USA). For each subject, the first four volumes of the fMRI series were discarded to allow for T1 equilibration effects. The pre-processing steps included correction for head motion, compensation for slice-dependent time shifts, normalization to the EPI template in MNI coordinates provided with SPM8, and smoothing with a 3D Gaussian Kernel with 8mm<sup>3</sup> full-width at half maximum. For each data set motion correction was checked to ensure that the maximum absolute shift did not exceed 2 mm and the maximum absolute rotation did not exceed 1.5°. The global temporal drift was removed using a 3rd order polynomial fit and the signal was regressed against the realignment parameters, and the signal averaged over whole brain voxels, to remove other potential sources of bias. Then, all images were filtered by a phase-insensitive band-pass filter (pass band 0.01-0.08 Hz) to reduce the effect of low frequency drift and high frequency physiological noise.

#### 2.4.3 Definition of Regions of Interest (ROIs) and Seed-based analyses

Based on the VBM results, the most affected cerebellar regions were identified and used as region of interest (ROI) in the seed-based analysis. The region of significant reduced GM volume was then extracted according to SUIT atlas template of the cerebellum (Diedrichsen et al., 2009) using



FSL command line from the FMRIB software library (FSL, [www.fmrib.ox.ac.uk/fsl/](http://www.fmrib.ox.ac.uk/fsl/)) and resliced into EPI standard space. The mean time course of the voxels within the affected ROI was calculated for every participant and used as a regressor in a 1st level SPM analysis, thus extracting the voxels in the whole brain showing a significant correlation with it. At second level, a two-sample t-test model was used to explore differences in connectivity between ASD and TDAs in the ROI. Results were considered significant at p-values <0.05 after FWE cluster-level correction (clusters formed with  $p < 0.005$  at uncorrected level). In order to remove the effect of confounding variables, the quantity of total brain GM volume, age and sex were entered in the analysis as covariates of no interest.

#### 2.4.4. Behavioral correlation with Regional GM

Based on VBM results, the cluster of significantly reduced GM in ASD patients was extracted and the GM volumes of the cluster were calculated for each subject. Spearman's correlations were then computed for the relationship between such volumes and ADOS and AQ total scores. Correlations significant at  $p < 0.05$  were reported.

### 3. Results

#### 3.1 Voxel-Based Morphometry

Voxel-wise analysis between modulated GM maps revealed a statistically significant GM ~~loss~~ reduction in the cerebellar cortex of ASD compared to TDAs. More specifically, a large cluster of significantly decreased GM volume (FWE  $p=0.05$ ) included regions in the right posterior cerebellar hemisphere, with prevalent involvement of the right Crus II. No regions of increased cerebellar

GM volume were found in ASD subjects compared to TDAs. Results of voxel-based morphometry are showed in Figure 1.

### 3.2 Functional connectivity seed-based analysis

No subject was excluded due to motion artifacts. According to voxel-wise analysis, the cerebellar Crus II was the most affected region in ASD compared to TDAs. In line with this finding, the right Crus II was used as ROI for the seed-based analysis. When comparing ASD with TDAs, a pattern of reduced functional connectivity was found between the cerebellar right Crus II and several regions in the contralateral cerebral cortex. Specifically, cluster-level peaks were found in the left middle and inferior temporal gyrus, left paracingulate cortex and frontal pole, also encompassing superior frontal gyrus, and bilateral precuneus regions. (Figure2A). Additionally, a pattern of increased FC was only found between the right Crus II and regions within the cerebellar cortex of ASD subjects, such as the right lobule V and VI and the left VI (Figure 2 B). MNI coordinates and peak-z scores are reported in the table 2A-B.

### 3.3 Behavioral correlation with GM volume

In line with voxel-wise analysis, the GM volumes of the cluster, extracted from the VBM results and centered in the right Crus II, were correlated with ADOS and AQ scores of ASD subjects. As showed by the Spearman correlation coefficients, we found a negative correlation between GM volumes in the Crus II and AQ scores ( $R^2 = -0.70$ ,  $p = 0.02$ ). No correlation was found between cerebellar GM reduction and ADOS scores ( $R^2 = -0.04$ ,  $p = 0.89$ ).

## 4. Discussion

The present MRI study provides evidence that adults with ASD present with a specific pattern of cerebellar alterations both in terms of morphometry and functional connectivity with cortical regions.

The results of the VBM analysis showed decreased GM volume in the posterior regions of the cerebellar cortex, mostly involving the right Crus II. When looking at functional connectivity, the

reduced cerebellar lobule was found to have decreased connectivity with frontal and temporal regions in the contralateral cerebral cortex and precuneus bilaterally, **while increased connectivity was found only with regions within the cerebellar cortex.** Additionally, a negative correlation between the degree of autistic traits, as measured by the AQ scores, and the GM volume of the right Crus II emerged, indicating that a greater degree of autistic traits was associated to lower GM volume in the cerebellar Crus II of ASD.

The cerebellum has been suggested to be part of the distributed neural circuits that may be dysfunctional in ASD (Bauman et al., 1985; Ritvo et al., 1986; Courchesne et al., 1994,1997). Indeed, a decreased cerebellar GM volume has been reported and consistent evidence converges into indicating the posterior regions of the cerebellum to be particularly affected. Results from a recent ALE meta-analysis of VBM studies revealed that several regions of the cerebellum show reduced GM in ASD, including vermis lobule IX, right Crus I, and left lobule VIIIB (Stoodley, 2014). Consistent with our results, the right Crus II has been also reported to show significantly reduced GM in individuals with ASD (Riva et al., 2013; D’Mello et al., 2015). Crus II is located in the lateral-posterior lobe of cerebellar hemispheres and is considered to be part of the social cognitive cerebellum (Van Overwalle et al., 2014, 2015a,b, 2016; Van Overwalle and Marien, 2016). **“Viral tract-tracing and human DTI studies link the posterior cerebellum (particularly Crus I/II, lobule IX, and the posterior vermis) with regions of the cerebral cortex involved in social processing and emotion, providing an anatomical substrate for cerebellar involvement in social cognition and affective regulation” (D’Mello et al., 2015).**

Accordingly, evidence from structural, functional and connectivity neuroimaging studies have shown the right Crus II to be related to more severe ASD impairment (D’Mello and Stoodley, 2015) and recently, lobular GM reductions in the Crus II of ASD subjects has been found to specifically correlate with ADOS scores (Riva et al., 2013; D’Mello et al., 2015).

In our study, no correlation was found between cerebellar volumetric alterations and ADOS scores. First of all, it has to be considered that both of the mentioned studies included **children (Riva et al.,**

2013; D'Mello et al., 2015) while we only included adults with mild ASD symptomatology and presenting normal or superior intellectual abilities.

Thus, inconsistent results may also occur due to the sample heterogeneity across studies. That being stated, we hypothesized that, in our sample, the reduced cerebellar volume in the Crus II is related to the clinical features of ASD, as represented by the AQ rather than ADOS scores. A possible explanation could be the administration of the instruments at two different times; the adults included in the present study received their official ASD diagnosis in childhood or in adolescence; the measures obtained at the same time (i.e AQ in this study) would be expected to be stronger than relations across time, and for this reason the traits measured with AQ might be more consistent and accurate than results from the ADOS. Alternatively, as reported by Charman et al. (2017), the lack of correlations could be due to the different type of measures: the ADOS is a diagnostic measure that is based on an observation and it taps variation in clinical level symptoms, whereas the AQ is a continuous self-report measure filled out by the subjects that evaluates the degree to which subjects with average intelligence show behavioral traits associated with ASD.

Previous functional magnetic resonance imaging (fMRI) studies have proposed that the ASD brain may be characterized by underconnectivity (Just et al., 2004; Muller, 2007; Muller et al., 2011) and that alterations in connectivity, rather than structural abnormalities in isolated brain regions, might be an even more important correlate of behavioral ASD-related deficits (Belmonte et al., 2004; Welchew et al., 2005). The cortical underconnectivity theory refers to underfunctioning of integrative circuitry and posits that “autism is a cognitive and neurobiological disorder marked and caused by underfunctioning among cortical areas that results in a deficit of integration of information at the neural and cognitive levels” (Just et al., 2004).

Across several studies, a complex neural network of cortical regions has been implicated in ASD. Specifically, cortical underconnectivity has been reported between dorsolateral prefrontal cortex and inferior parietal lobule (Just et al., 2007), frontal cortex and fusiform gyrus (Koshino et al., 2008), medial frontal and temporo-parietal regions (Castelli et al., 2002; Kana et al., 2009).

Supporting our results, a recent fMRI study described a reduction of connectivity specifically between the left temporal area and the superior and medial frontal gyrus (Hoffman et al., 2016), regions that have been implicated in higher mental processes strictly related to social functioning, such as mentalizing and reward anticipation (Amodio and Frith, 2006).

These regions are functionally connected with the cerebellum, and specifically with the Crus II (Mars et al., 2012) where a concentration of supramodal projections converges (O'Reilly et al., 2010). Further support to our findings comes from recent **fMRI data** on healthy subjects demonstrating functional connectivity between the social mentalizing cerebellum, in particular including the right posterior cerebellar lobules, and the social mentalizing areas in the cerebrum (Van Overwalle and Marien, 2016). Specifically, the mean social cerebellar peak was computed across different fMRI studies and resulted in the mean MNI coordinates of 25 -75 -40 converging onto right cerebellar Crus II. It is worth noting that in the present study peak voxel of cerebellar **GM reduction** in ASD was centered at very close MNI coordinates (29 -73 -43) in the right Crus II. This similarity of results strongly reinforces the significance of our conclusions and gives **further support** to the idea that the right Crus II plays a specific role in social mentalizing functions, **as previously proposed by other authors (Van Overwalle et al. 2014, 2015a,b, 2016; Hoche et al. 2016).**

A recent resting-state fMRI study specifically investigated cerebro-cerebellar functional connectivity in children with ASD. Underconnectivity in supramodal regions of prefrontal cortex (PFC), posterior parietal cortex (PPC) and middle temporal gyrus (MT) has been detected predominantly with the posterolateral neocerebellum and primarily with the lobule VII, also encompassing the Crus II (Khan et al., 2015).

In light of these observations, our results provide additional insight into understanding the neural substrate of ASD. By using an integrated approach in adults with high-functioning ASD, the present study shows a specific cerebellar pattern of structural abnormality and functional underconnectivity that is largely consistent with existing anatomical and functional findings in healthy subjects and

different ASD populations. The idea is that the cerebral areas that receive projections from the affected region in the cerebellum can be selectively impaired due to a loss or dysfunction of the cerebellar modulation. It is worth noting that a pattern of increased FC was only found within the cerebellum itself, specifically between the cerebellar ROI in the right Crus II and cerebellar lobules V-VI. We speculate that these findings are probably due to adaptive compensatory mechanisms induced in response to the functional alteration of the Crus II. Overall, we advance that the cerebellar abnormality in the right Crus II, as showed by VBM analysis, may selectively affect long-distance cerebral regions relevant to social processing thus impairing optimization of functions in specific cerebello-cerebral modules clearly related to ASD core traits. In particular, Crus II has been shown to modulate the activity of DMN, a network strictly related to social cognition processing and including a set of cortical and subcortical brain structures (Raichle et al., 2001). Consistent with this evidence, in ASD individuals reduced functional connectivity was found between Crus II and key cortical default mode regions, such as superior frontal gyrus, temporal lobe and precuneus. Furthermore,

Interestingly, the correlation between reduced GM volume in the Crus II and autistic traits, as expressed by the AQ scores, provide additional support to our hypothesis.

An important issue that needs to be discussed concerns with the gender distribution within our ASD group, since that the epidemiology of autism typically indicates a male prevalence (Fombonne, 2005). Although a gender bias is very likely to be genuine, the traditionally accepted 4–5:1 male prevalence has been recently questioned. Indeed, It has been suggested that the male prevalence may be in part due to the “underrecognition of females (particularly higher-functioning), ascertainment bias, and issues of diagnostic instruments” (Lai et al., 2015), as well as to the fact that the diagnosis is later in females than in males (Beeger et al., 2015; Giarelli et al., 2010). In particular, higher-functioning females might be under- or misrecognized until later in adolescence or adulthood. With regard to this, it has to be considered that we only included high functioning

adult subjects and some studies tend to find no sex/gender differences in this kind of ASD population (Lai et al., 2011; Hofvander et al., 2009; Lugnegård et al., 2011).

Finally, in analyzing the present results, it has to be considered that the small number of the patients taking part in the study could limit the strength of the conclusions. This limitation is due to the strict inclusion criteria that clearly affected the inclusion rate. In spite of this, the statistically high significance of our data and the consistence with the more recent ASD studies, strongly reinforces the relevance of the results supporting the crucial role of **the** cerebellum in social cognition via interaction with cortical mentalizing network and providing additional evidence with a selected ASD population.

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**Conflict of interest:** The authors declare that they have no conflict of interest.

## **Author Contribution**

G. Olivito, S. Clausi and M. Leggio contributed to the ideas and formulation of goals and aims. G. Olivito, M. Lupo, S. Clausi and M. Leggio contributed to data curation, including the management of research data; G. Olivito and M. Cercignani contributed to the formal analysis including the preprocessing of MRI data and application of statistical, or other formal techniques to analyze or synthesize study data; M. Lupo, R. Baiocco and S. Clausi, contributed to conducting the research and investigation process, performing the experiments, or data/evidence collection; M. Leggio, F. Laghi, and M. Bozzali contributed to the management, coordination responsibility and supervision of the research activity planning and execution; G. Olivito and M. Leggio contributed to Original Draft Preparation specifically writing the initial draft.

All authors contributed to review and editing of the original draft, including pre- or post-publication stages.



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**Table 1. Demographic and clinical characteristics of ASD subjects.**

CASE CODE	AGE	EDUCATION	GENDER	ADOS SCORES	AQ SCORES
ASD-1	21	13	F	16	36
ASD-2	17	12	M	13	24
ASD-3	31	13	F	13	42
ASD-4	33	13	F	7	42
ASD-5	29	18	M	11	42
ASD-6	18	12	M	9	30
ASD-7	23	13	M	7	42
ASD-8	18	12	F	7	42
ASD-9	19	14	F	12	46
ASD-10	45	13	M	13	47
Mean(SD)	25.4(9)	13.3(1.7)	-	10.8(3.1)	39.3(7.2)

The table reports for each subjects age, education, gender, ADOS total scores, and AQ scores. Mean scores and standard deviations (SD) are also reported. F:female; M:male.

**Table 2. Functional Connectivity changes of the right Crus II**

Regions	Size (NoV)	Side	Coordinates (mm)			Peak Z-score
			x	y	z	
A)						
Middle Temporal gyrus	440	L	-56	-18	-10	3.50
Inferior Temporal gyrus			-48	-44	-12	3.41
Paracingulate cortex	381	L	-8	56	6	3.53
Frontal pole			-6	62	14	3.37
Precuneus	352	L	-2	-70	32	3.36
		R	10	-74	34	3.01
B)						
Lobule V	618	R	26	-42	-20	3.77
Lobule VI		L	-24	-46	-30	3.67
Lobule VI		R	26	-48	-26	3.40

A) Pattern of functional underconnectivity between the right Crus II and the cerebral cortex regions in ASD subjects ;

B) Pattern of functional overconnectivity between the right Crus II and cerebellar regions in ASD subjects

**Note:** MNI coordinates (X, Y, Z in the Montreal Neurological Institute space) and peak Z-score of the peak voxel showing greatest statistical differences in a cluster. NoV= number of voxels.

## Figure Captions.

### **Figure 1.** Between groups voxel-based comparison of cerebellar **GM volume**.

Cerebellar regions showing patterns of significantly reduced GM in ASD compared to TDAs are reported and superimposed on axial ( $z=38$ ), coronal ( $y=20$ ) and sagittal ( $x=91$ ) sections of the Spatially Unbiased Infratentorial Template (SUIT) (Diedrichsen et al., 2009). Statistical significance was found at cluster level (FWE= 0,05; cluster size: 1629 with prevalent involvement of the right Crus II (peak voxel centered at  $x=29$   $y=-73$   $z=-43$ , and  $x=17$   $y=-79$   $z=-29$  4 in the Montreal Neurological Institute space). R=Right; L=Left

### **Figure 2. Functional connectivity patterns of the Right Crus II.**

Functional under- (A) and over-connectivity (B) of the right Crus II in ASD compared to TDAs are shown in coronal (left column), sagittal (central column) and axial slices (right column). Clusters of significantly decreased functional connectivity (in blue) (FWE =0.05) included several regions in the frontal, temporal, and parietal cortices. A cluster of significantly increased functional connectivity (in red) (FWE =0.05) included regions within the cerebellar cortex (peak voxel in the lobules V-VI). X, Y, Z in the Montreal Neurological Institute space (left and right hemispheres are on the left and right side of the figure, respectively). See table 2 A/B for details.